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Original article

Synthesis and broad spectrum antiviral evaluation of *bis*(POM) prodrugs of novel acyclic nucleosides

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ABSTRACT

A series of seventeen *hitherto* unknown ANP analogs bearing the (*E*)-but-2-enyl aliphatic side chain and modified heterocyclic base such as cytosine and 5-fluorocytosine, 2-pyrazinecarboxamide, 1,2,4-triazole-3-carboxamide or 4-substituted-1,2,3-triazoles were prepared in a straight approach through an olefin acyclic cross metathesis as key synthetic step.

All novel compounds were evaluated for their antiviral activities against a large number of DNA and RNA viruses including herpes simplex virus type 1 and 2, varicella zoster virus, feline herpes virus, human cytomegalovirus, hepatitis C virus (HCV), HIV-1 and HIV-2. Among these molecules, only compound **31** showed activity against human cytomegalovirus in HEL cell cultures with at EC_{50} of ~10 μM. Compounds **8a**, **13**, **14**, and **24** demonstrated pronounced anti-HCV activity without significant cytotoxicity at 100 μM.

1. Introduction

The discovery by A. Holý and E. De Clercq in 1986 of broad-spectrum antiviral activity of (*S*)-HPMPA [9-[3-hydroxy-2-(phosphonomethoxy)propyl]adenine] and PMEA [9-[2-(phosphonomethoxy)ethyl]adenine] led to a new family of nucleotides designated as acyclic nucleoside phosphonates (ANP) [1–4],. ANPs are nucleotide analogs that are characterized by the presence of a phosphonate group linked to a pyrimidine or purine base through an aliphatic linker. Three of these are approved drugs for the treatment of severe/fatal infectious diseases and represent three different types of ANPs: (i) HPMP derivatives such as (*S*)-1-[3-hydroxy-2-(phosphonomethoxy)propyl]cytosine (HPMPC, cidofovir (Vistide®)] which is approved for the treatment of cytomegalovirus (CMV) retinitis in AIDS patients [5]; (ii) PME derivatives such as PMEA [adefovir (in its oral prodrug form, adefovir dipivoxil (Hepsera®)] for the treatment of hepatitis B virus infections [6], and (iii) PMP derivatives such as PMPA [tenofovir (in its oral prodrug form, tenofovir disoproxil fumarate (Viread®)] is used for the treatment of HIV infections (AIDS) and hepatitis B virus [7]. From these data, it appears that small chemical alterations in the acyclic side-chain lead to marked differences in antiviral activity and the spectrum of activity of acyclic nucleoside phosphonates against various classes of viral agents [1].

Thus, the synthesis and biological evaluation of a large panel of ANPs were systematically investigated as potential antiviral compounds [1]. In our search for antiviral compounds, we synthesized a new class of acyclic nucleoside phosphonates based on a 4-phosphono-but-2-en-1-yl base motif in which the oxygen heteroatom has been replaced with a double bond having *trans* stereochemistry [8]. We have shown that this modification allows

Abbreviations: VZV, varicella zoster virus; VV, vaccinia virus; HSV, herpes simplex virus; VSV, vesicular stomatitis virus; DNA, deoxyribonucleic acid; RNA, ribonucleic acid; CC_{50}, compound concentration affording 50% inhibition of cell growth; EC_{50}, compound concentration affording 50% inhibition of the viral cytopathicity; MCC, minimum cytotoxic concentration required to afford a microscopically detectable alteration of cell morphology; MDCK, Madin–Darby canine kidney.

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mimicry of the three-dimensional geometry provided by the backbone of PMEA, PMPA, and CDV while maintaining an electronic contribution similar to that brought by the oxygen atom [8]. Several new derivatives are efficiently activated by human thymidylate kinase (hTMPK), and the best substrates were converted to *bis*-(pivaloyloxymethyl)ester phosphonate prodrugs and found to be active against several herpes viruses in cell culture.

On the basis of these findings, it was interesting to design and synthesize *hitherto* unknown ANP analogs bearing the biolabile phosphonate (*E*)-but-2-enyl aliphatic side-chain and a series of modified heterobases selected from the literature as lead nucleobases with antiviral properties, such as cytosine and 5-fluoro cytosine, 2-pyrazinecarboxamide, 1,2,4-triazole-3-carboxamide or 4-substituted-1,2,3-triazoles (Fig. 1). From a chemical synthesis point of view, the strategy based on olefin cross metathesis we have developed to obtain a large library of (*E*)-4-phosphono-but-2′-en-1′-yl pyrimidine nucleosides [9–11].

2. Results and discussion

2.1. Chemistry

First, we turned our attention to the synthesis of cytosine derivatives as cytosine modified nucleosides form a prolific family of antitumor and antiviral agents [12]. As it could be excepted, even if the ruthenium carbene complex **3** (Grubbs–Nolan catalyst 2nd generation) is less affected by free amine and nitrogen-containing groups than the Grubbs's 1st generation catalyst [13], the cross-metathesis reaction between unprotected N^1-crotylated cytosine **2** and *bis*-(POM) allylphosphonate **1** failed (Scheme 1).

The successful cross-metathesis occurred with protected N^1-crotylated cytosines **6a**, **b**. Thus, cytosine **4a** and 5-fluorocytosine **4b** were converted to their N^4-*bis*-Boc cytosine derivatives **5a**, **b**, respectively, through a *N*-peracylation followed by subsequent and regioselective N^1 deprotection by a saturated solution of $NaHCO_3$ in methanol [14,15]. Crotylation of the N^1 position of **5a**, **b** using Cs_2CO_3 and crotyl bromide afforded the desired compounds **6a** (85%) and **6b** (81%). Compound **6a**, **b** were then engaged in the olefin cross metathesis reaction with *bis*(POM)-allylphosphonate **1** using 5 mol% of the (NHC)Ru=CHR Nolan's catalyst, $Cl_2(PCy_3)$(IMes)Ru

(CHPh) (**3**), in dry CH_2Cl_2 (0.1 M) at reflux to afford (*E*)-N^1-(4′-*bis*(POM)-phosphinyl-2′-butenyl)-*bis*-Boc-cytosine **7a**, **b** in moderate yields (43% (for R = H) and 26% (for R = F)). The removal of the Boc group requires oftentimes harsh conditions (e.g. trifluoroacetic acid, trimethylsilyl iodide, hydrochloric acid in ethyl acetate, potassium carbonate, etc.) that are not compatible with the POM moiety. However, Hwu et al. [16] have reported an efficient and milder selective Boc deprotection under neutral conditions using ceric ammonium nitrate (CAN) that is consistent with the stability of the phosphonate biolabile group. Thus, protected ANP **7a**, **b** were reacted with a catalytic amount of ceric ammonium nitrate (CAN) (20 mmol%) in CH_3CN–MeOH (1:1) to give the expected compounds **8a**, **b**, respectively, in moderate yield, with no observed removal of the POM moiety (Scheme 2).

We turned then our attention to the synthesis of the pyrazinecarboxamide derivative **14** since, among the modified nucleobases, a series of pyrazinecarboxamide derivatives (including T-705, favipiravir) developed by Furuta et al. [17,18] have demonstrated good activity in various RNA viral infections. In a first attempt, we struggled to introduce the pyrazine moiety through direct *N*-alkylation of **10** with the corresponding (*E*)-4-phosphono-but-2′-en-1′-yl bromide (**9**), in the presence of K_2CO_3 in anhydrous DMF, (Pathway A). Unfortunately only the *bis*(POM)-but-1,3-dienyl phosphonate **9′** resulting from undesired bromine elimination was obtained, (Scheme 3). Thus, we decided to reach the pyrazine phosphonate analogs **13**, **14** following the same strategy developed for the cytosine derivatives, (Pathway B). Starting from the 3-hydroxypyrazine-2-carboxamide **10**, the N^1-Crotyl-3-oxo-pyrazine-N^3-*bis*-Boc-carboxamide **11** was obtained in 49% yield, via the two step crotylation/*N*-Boc-protection. Next, N^1-crotyl-3-oxo-pyrazine-N^3-*bis*-(Boc)-carboxamide **11** in CH_2Cl_2 was treated in the presence of *bis*(POM)-allylphosphonate **1** and (NHC)Ru=CHR Nolan's catalyst **3** to give the desired product **12** in 22% yield. To obtain the carboxamide **14**, we first applied our previous described methods, using ceric ammonium nitrate and CH_3CN–MeOH (1:1). Surprisingly, the methylester **13** was isolated as the major compound in 47% yield. Thinking that the presence of the undesired product **13** was due to the use of methanol as co-solvent, the preparation of the desired amide **14**, was achieved in CH_3CN using ceric ammonium nitrate in 25% yield.

Based on the above results, we extended this approach to the formation of a ribavirin analog bearing the 1,2,4-triazole derivative [19,20]. Starting from **15**, the protection of both nitrogens provided compound **16**, in quantitative yield, which is directly used in the next step. An attempt to selectively Boc deprotect at the N^1 position

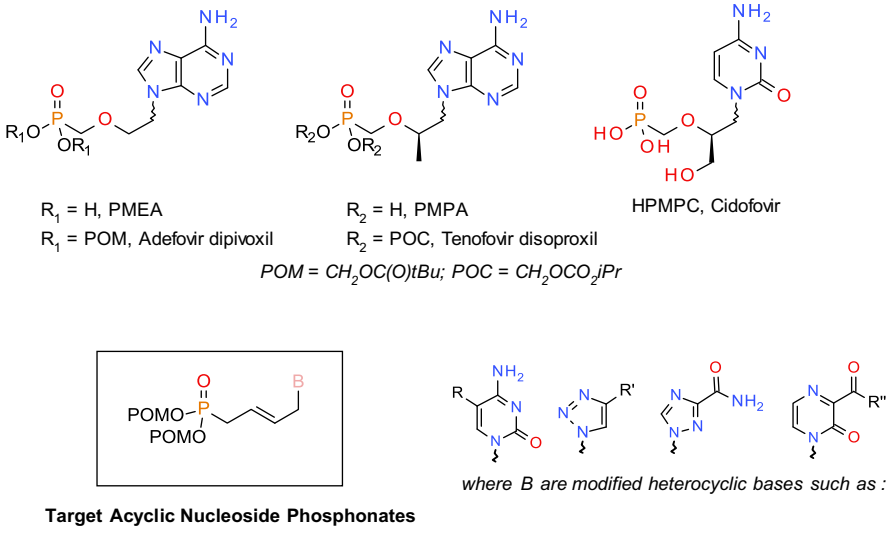

Fig. 1. Structure of selected ANPs and target derivatives.